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PROHEXADIONE CALCIUM **Reproduction Study (§83-4a)**

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DATA EVALUATION RECORD

STUDY TYPE: Multigeneration Reproduction Study - Rat
OPPTS Number: 870.3800 OPP Guideline Number: §83-4a

DP BARCODE: D246707 SUBMISSION CODE: S543930
P.C. CODE: 069089 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Prohexadione calcium (91.9-93.8% a.i.)

SYNONYMS: Cyclohexanecarboxylic acid, calcium salt of 3,5-dioxo-4-propionyl-cyclohexane-1-carboxylic acid, BX-112

CITATION: York, R.G. (1992). Two-Generation Dietary Reproduction Study in Rats with BX-112. International Research and Development Corporation, Mattawan, Michigan. Laboratory Project ID 442-037. October 6, 1992. MRID 44457764. Unpublished.

York, R.G. (1990). Reproductive Range-Finding Study in Mated Rats with BX-112. International Research and Development Corporation, Mattawan, Michigan. Laboratory Project ID 442-035. August 9, 1990. MRID 44457763. Unpublished.

SPONSOR: BASF Corporation, Agricultural Products, P.O. Box 13528, Research Triangle Park, North Carolina

EXECUTIVE SUMMARY: In a 2-generation reproduction toxicity study (MRIDs 44457764 and 44457763), prohexadione calcium (91.9-93.8% a.i.) was administered continuously in the diet to CD rats (26/sex/dose) at nominal dose levels of 0, 500, 5,000 or 50,000 ppm. Based on the concentration analyses, achieved doses were calculated by the reviewers as 0, 355, 3,850, or 38,500 ppm (equivalent to 0, 35.5, 385.0, or 3,850.0 mg/kg/day, respectively, in the P animals and F_1 animals). Exposure to P animals began at 36 days of age and lasted for 56 days prior to mating. F_1 pups selected (26/sex/dose) to produce the F_2 generation were exposed to the same dosage as their parents beginning at postnatal day (PND) 21 and continuously throughout the rest of the study. F_1 animals were administered the test article for approximately 63 days prior to mating to produce the F_2 litters. Mating to produce a second F_{2b} generation was not performed. Exposure of all animals to the test material was continuous throughout the study.

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There were no treatment-related clinical signs observed in the P or F_1 animals. There were no changes of toxicological concern in food consumption, reproductive performance, gross pathology, testes weights, or histopathology.

In the mid-dose group, two P males and one F_1 female died.

In the high-dose group, two P males died and one female was euthanized *in extremis*. F_1 males had decreased body weights throughout treatment (↓11-30%, $p \leq 0.01$). F_1 females had reduced body weight for weeks 4-12 and week 15 (↓7-33%, $p \leq 0.05$ or 0.01). Decreases in body weight gains occurred in P females for the overall gestation period (↓17%, $p \leq 0.05$).

The NOAEL for parental systemic toxicity is 355 ppm (35.5 mg/kg/day). The LOAEL for parental systemic toxicity is 3850 ppm (385 mg/kg/day) in males and females based on increased mortality.

No effects of toxicological concern were observed in the reproductive parameters or function of the adults. **The reproductive toxicity NOAEL is ≥38,500 ppm (3850 mg/kg/day). The reproductive toxicity LOAEL is >38,500 ppm (3850 mg/kg/day).**

No effects of toxicological concern on mortality or clinical signs were observed at any time in the F_1 and F_2 litters. There were no treatment-related findings at necropsy in the F_1 or F_2 pups. In the high-dose groups, body weights were decreased ($p<0.05$ or 0.01) in F_1 pups throughout lactation (↓8-26%) and in F_2 pups at LD 14 and 21 (↓9-23%). Body weights were also decreased ($p<0.05$) in the mid-dose groups compared to concurrent controls in F_1 pups on LD 14 (↓5%) and F_1 males on LD 21 (↓5%). No observations of toxicological significance were made at the low-dose.

The NOAEL for offspring toxicity is 3850 ppm (385 mg/kg/day). The LOAEL for offspring toxicity is 38,500 ppm (3850 mg/kg/day) in males and females based on decreased pup body weight.

This reproductive study is determined to be **acceptable (§83-4(a), reproduction)** and does satisfy the guideline requirement for a multigenerational reproductive toxicity study in rats.

COMPLIANCE: Signed and dated Quality Assurance, Data Confidentiality, GLP, and Flagging statements were provided.

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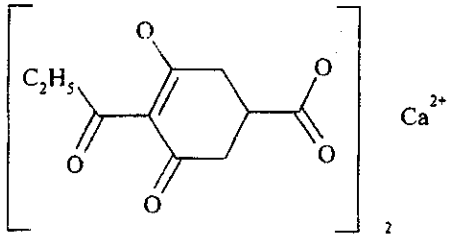
I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: BX-112
   Description: Tan powder
   Lot/Batch #: G14-12 and G14-13
   Purity: 91.9-93.8% a.i.
   Storage: 4°C in the dark
   CAS #: 127277-53-6
   Structure:



2. Vehicle: Diet

3. Test animals: Species: Rat
   Strain: Crl:CD VAF/Plus®
   Age at start of dosing: P - 36 days; F_1 - approximately 21 days
   Weight at start of dosing:
   (P) Males: 98-142 g; Females: 126-150 g
   (F_1, group means) Males: 43.7-59.0 g; Females: 41.8-55.5 g
   Source: Charles River Breeding Laboratories, Inc. Portage, MI
   Housing: Stainless-steel wire-mesh during premating (1/cage) and mating (1 male and 1 female/cage), and in plastic cages with solid bottom and bedding during gestation and lactation (1 dam and litter/cage)
   Diet: Purina Certified Rodent Chow #5002 (Purina Mills, St. Louis, MO), ad libitum
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 18-24°C
   Humidity: 32-65%
   Air changes: Not reported
   Photoperiod: 12 hours light/12 hours dark
   Acclimation period: 8 days
   Study Duration (in life dates): 6/12/90 to 2/7/91

B. PROCEDURES AND STUDY DESIGN

1. Mating procedure: One male was caged with one female from the same test group for 21 days or until sperm was observed in a vaginal smear. This day was designated gestation day (GD) 0. Females with no evidence of mating were maintained for 25 days to

determine pregnancy status. Non-pregnant females were sacrificed and necropsied at that time.

2. Study schedule: Starting at 36 days of age, P animals were given test article diet formulations for 56 days prior to mating to produce the F_1 litters. At postnatal day (PND) 21. F_1 animals were selected to become the F_1 parents of the F_2 generations and were given the same concentration test formulation as their dam. F_1 animals were given test formulations for a minimum of 63 days prior to mating the first time to produce the F_2 litters. Mating to produce a second F_2 generation was not performed. F_2 litters were necropsied at weaning. Exposure of all animals to the test material was continuous throughout the study.

3. Animal assignment: P animals were randomly assigned using a body weight stratification technique to test groups as seen in Table 1. F_1 animals were randomly selected from the weaned litters to become the F_1 parents.

Table 1. Animal assignment

Test Group	Dose (ppm)[a]	Achieved Dose (ppm)[b]	Animals/group			
			P Males	P Females	F_1 Males	F_1 Females
Control	0	0	26	26	26	26
Low	500	355	26	26	26	26
Mid	5,000	3,850	26	26	26	26
High	50,000	38,500	26	26	26	26

a Diets were administered from the beginning of study until sacrifice.
b Achieved dosages were calculated by the reviewers based on concentration analyses averages presented in Table 3, page 48 of the study report.

4. Dose selection rationale: The test concentrations were selected based on the results of a range-finding study in rats (MRID 44457763). In this 1-generation reproduction toxicity study, prohexadione calcium was administered continuously in the diet to CD rats (8/sex/dose) at dose levels of 0, 400, 2,000, 10,000, or 50,000 ppm. Animals were mated after 28 days of exposure. Males were sacrificed and necropsied following mating; females and their litters were sacrificed and necropsied on lactation day (LD) 21. At 50,000 ppm, 1 male rat died of unknown causes; thickened stomach mucosa was observed in 3/8 males; body weights were decreased ($p<0.05$ or 0.01) in males at week 1 (↓14%), females at weeks 1 and 3 (↓5-8%), and pups at LD 21 (↓22%, p=not statistically significant); food consumption was decreased ($p<0.05$) in males (g/kg/day) at weeks 3

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and 4 (111-15%) and females (g/animal/day) at week 1 (112%). Based on the results of this study, 50,000 ppm was chosen as the high-dose; 5,000 and 500 ppm were chosen as the mid- and low-doses, respectively.

5. Dosage preparation and analysis: Formulations were prepared weekly by mixing appropriate amounts of the test article with the diet. For homogeneity analyses, 10 subsamples of each diet were analyzed prior to the study initiation. For stability analyses, a composite sample was taken at the time of homogeneity testing and stored "under normal laboratory conditions for 10 days"; it was not stated whether the sample was refrigerated or protected from light. For concentration analyses, formulations from weeks 1 and 3 were analyzed.

   Results - Homogeneity Analysis (range as % of nominal ± relative standard deviation): 72-93 ±3.3-8.5%.

   Stability Analysis: (as % of day 0): 104%

   Concentration Analysis (range as % of nominal): 70-81%. BX-112 was not detected in control samples.

   The analytical data indicated that the test article formulation was stable. Based on concentration analyses averages, achieved dosages for the nominal dosages of 0, 500, 5,000, and 50,000 ppm were calculated by the reviewers as 0, 355, 3,850, and 38,500 ppm, respectively.

6. Dosage administration: Formulations were administered continuously in the diet.

C. OBSERVATIONS

1. Parental animals: All parental animals were observed twice daily for morbidity or mortality and clinical signs. Males were weighed weekly during the study. Females were weighed weekly during premating, on GDs 0, 6, 15, and 20, and on LDs 0, 7, 14, and 21. Body weight gains were calculated for the gestation and lactation periods only. Food consumption was measured weekly for both sexes during the premating period and for males during the postmating period until necropsy. Maternal food consumption was recorded for intervals during gestation and lactation corresponding to the days on which body weights were measured. These data were then used to calculate the average test substance intake for individuals and treatment groups. Water consumption was recorded for the same intervals as food consumption.

2. Litter observations: Litters were examined daily for mortality, sex, and clinical signs. The following litter observations (X) were made (Table 2):

Table 2. F_1 litter observations[a]

Observation	Time of observation (lactation day)						
	Day 0	Day 1	Day 4[b]	Day 4[c]	Day 7	Day 14	Day 21
Number of live pups	X	X	X	X	X	X	X
Pup weight	X		X	X	X	X	X
Behavior abnormalities	X	X	X	X	X	X	X
Number of dead pups	X	X	X		X	X	X
Sex of each pup	NR	NR	NR	X	NR	NR	X

a Data extracted from the study report, pages 22, 93, and 97.
b Before standardization (culling)
c After standardization (culling)
NR Not reported

On PND 4, litters were standardized to a maximum of 8 pups/litter with 4/sex/litter, as nearly as possible. Excess pups were sacrificed and given a complete gross necropsy. Abnormal tissues were preserved. Pups that died or were sacrificed prematurely received a necropsy.

3. Sexual development: Sexual development of pups was not assessed.

4. Postmortem observations:

1) Parental animals: P sires were sacrificed 3 weeks after the completion of mating. F_1 sires were sacrificed after the completion of parturition in the F_1 females. Non-mated females were sacrificed approximately 25 days after the end of the mating period. P and F_1 dams that littered were sacrificed at weaning. All parental animals were subjected to a complete external and internal postmortem examination. The uteri of all non-pregnant females were stained using 10% ammonium sulphide to confirm pregnancy status. The animals were weighed, and the following organs or tissues were weighed (XX) and/or preserved (X) in neutral buffered formalin for possible future examination:

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	Adrenals		Brain
	Kidneys		Spleen
	Liver		Thymus
X	Prostate	XX	Testes/epididymis
X	Seminal Vesicles		Cervix
X	Ovaries	X	Uterus
X	Vagina	X	Pituitary
	Oviducts	X	Stomach, glandular
X	Stomach, nonglandular	X	Gross lesions

Slides prepared from these tissues were examined for all high-dose and control adults, and from any animals which died or were euthanized.

2) Offspring: Preweaning pups that died, were euthanized, or culled at PND 4 or at weaning were necropsied. Abnormal organs or tissues were preserved. The F_2 pups were sacrificed at weaning and necropsied.

D. DATA ANALYSIS

1. Statistical analyses: All collected data were subjected to routine appropriate statistical procedures. Copulatory, fertility, and gestation indices were tested for significant differences using the Pearson Chi-square test with Yates' correction and/or Fisher's exact probability test. Pup survival indices were tested by the Mann-Whitney U-test. Parental body weight and food consumption, and pup viability and body weight were tested by a one-way analysis of variance (ANOVA), Bartlett's test for homogeneity of variances and t-test using Dunnett's multiple comparison tables, or heterogenous Student's t-test.

2. Indices:

Reproductive indices: The following reproductive indices as presented in the study report were calculated for the P and F_1 adults:

male/female copulation index = # of animals mated/# of animals paired x 100%

male/female fertility index = # of females pregnant/# of males/females mated x 100%

gestation index = # of females with live pups/# of females pregnant x 100%

Offspring viability indices: The following viability indices as presented in the study report were calculated for the F_1 and F_2 litters:

live birth index = # of live pups alive at birth/total # of pups born x 100%

day 4 viability index = # of live pups at Day 4 (precull)/# of pups born alive x 100%

day 7 viability index = # of live pups at Day 7/# of live pups at Day 4 (postcull) x 100%

day 14 viability index = # of live pups at Day 14/# of live pups at Day 7 x 100%

day 21 viability index = # of live pups at Day 21/# of live pups at Day 14 x 100%

weaning index = # of live pups at Day 21/# of live pups at Day 4 (postcull) x 100%

3. Historical control data: Historical control data were provided for comparison with concurrent controls.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs: In the P animals, two mid-dose males, two high-dose males, and one high-dose female died or were euthanized *in extremis*. In the F_1 animals, one mid-dose female died. There were no other premature deaths. There were no treatment-related clinical signs observed in the P or F_1 animals.

2. Body weight, body weight gain, and food consumption: No body weight changes of toxicological concern were seen in the P animals. High-dose F_1 males had decreased body weights throughout treatment (↓11-30%, $p \leq 0.01$, Table 3a). High-dose F_1 females had reduced body weight for weeks 4-12 and week 15 (↓7-33%, $p \leq 0.05$ or 0.01). Additional decreases ($p \leq 0.05$) in body weight occurred at other timepoints and in other groups, but these decreases were sporadic or too small to be of toxicological concern.

Table 3a. Selected mean body weights (g); P generation females during gestation; F_1 generation males during premating, mating, and postmating and females during premating.[a]

Study Days	Dose (ppm)			
	0	500	5,000	50,000
P Generation Females - Gestation				
Day 6	311	319	323	313
Day 15	353	350	359	350
Day 20	406	407	415	391
F_1 Generation Males - Premating, Mating, and Postmating				
Week 4	105	100	102	74**
Week 10	427	421	424	368**
Week 21	634	604	620	559**
F_1 Generation Females - Throughout Study				
Week 4	98	93	90**	66**
Week 10	257	251	248	238**
Week 15	318	314	314	290*

a Data extracted from the study report Tables 7 and 9, pages 55 and 59 and 60.
** Significantly different from controls at $p \leq 0.01$.

Decreases in body weight gains occurred in high-dose P females for the overall gestation period (↓17%, $p \leq 0.05$; Table 3b). No differences were observed in the F_1 dams.

Table 3b. Selected mean body weight gains (g); P generation females during gestation.[a]

Gestation Days	Dose (ppm)			
	0	500	5,000	50,000
P Generation Females - Gestation				
Day 0-6	29	33	31	27
Day 6-15	42	31	36	37
Day 15-20	53	58	56	41
Day 0-20	124	121	124	103*

a Data extracted from the study report Table 7, page 56.
* Significantly different from controls at $p \leq 0.05$.

There were no changes in food consumption of toxicological concern observed in any of the groups. Food consumption decreased in the first 2-3 weeks after weaning in the high-dose F_1 males and females (↓16-33%, $p \leq 0.01$), and was similar to controls for the

remainder of the study. These decreases were considered to be due to the unpalatability of the diet formulation. Small or sporadic increases and decreases ($p<0.05$ or 0.01) in food consumption occurred at different time points and in other dose groups, but these were not considered to be of toxicological concern.

3. Compound intake: The mean intake of BX-112 (mg/kg/day) was calculated using the food consumption and diet concentration (Table 4).

Table 4. Mean compound intake (mg/kg/day).[a]

Treatment group	Dose (ppm)			
	0	500	5,000	50,000
P males	0	34.92	349.19	3693.45
P females	0	46.02	466.84	4752.46
F_1 males	0	38.07	385.55	4251.13
F_1 females	0	48.15	483.40	5304.04

a Data were calculated by the reviewers from data in the study report Tables 18 and 21, pages 73, 74, 77, and 78.

4. Water consumption: Water consumption was increased in high-dose P (↑1-28%) and F_1 females (↑4-23%) and P males (↑9-29%) for the majority of the weeks of study. The increase in the F_1 females at week 20 (↑138%) was attributed to the low water consumption of the control females. These data were not analyzed for statistical significance and the differences were not sufficiently large to be considered of toxicological concern.

5. Reproductive function:

   a. Estrous cycle length and periodicity: No observations were made pertaining to the number or length of estrous cycles during the premating period in F_1 females.
   b. Sperm and male reproductive organ measures in P and F_1 males: It was stated that sperm were present, motile and morphologically normal in the P males that were tested for spermatogenesis. No observations were made pertaining to the sperm and male reproductive organ measures of the F_1 males.
   c. Sexual maturation (F_1): No observations were made pertaining to the sexual maturation.

6. Reproductive performance: There were no differences of toxicological concern observed in reproductive performance (Table 5). A slight reduction (↓9-10%) occurred in the P male and female fertility indices, but no effect was seen in the F_1 generation, and

therefore, this finding was not considered to be of toxicological concern.

Table 5. Reproductive performance of P and F_1 sires and dams.[a]

Observation	Dose (ppm)			
	0	500	5,000	50,000
P Generation - Litter F_1				
Male Copulation Index-%	100	100	92.0	100
Female Copulation Index-%	100	100	92.3	100
Male Fertility Index-%	92.3	88.5	87.0	83.3
Female Fertility Index-%	92.3	88.5	83.3	84.0
Copulatory Interval (days)	2.5	4.5	3.5	3.5
Gestation Index-%	100	100	100	95.2
Gestation Length (days)	22.2	22.1	22.0	22.0
Number of Litters	24	23	20	21
F_1 Generation - Litter F_2				
Male Copulation Index-%	92.3	73.1	84.6	92.3
Female Copulation Index-%	92.3	73.1	84.6	92.3
Male Fertility Index-%	84.6	69.2	84.6	88.5
Female Fertility Index-%	91.7	94.7	100	95.8
Copulatory Interval (days)	3.6	3.4	3.9	3.5
Gestation Index-%	100	100	90.9	100
Gestation Length (days)	22.2	21.9	21.9	22.1
Number of Litters	22	18	22	23

a Data extracted from the study report Tables 30 and 33, pages 89, 90, and 94.

7. Parental postmortem results

a) Gross pathology: Treatment-related pathological findings were limited to trace to mild red foci in the glandular stomach of the high-dose P males (10/24 treated vs 0/26 controls) and trace to moderate thickening of the junction of the glandular and nonglandular stomach (the limiting ridge) in the high-dose P males (9/24 treated vs 0/26 controls) and females (16/25 treated vs 0/26 controls), mid-dose P females (9/26 treated), high-dose F_1 males (23/26 vs 0/26 controls), and the high-dose F_1 females (25/26 treated vs 0/26 controls). These changes were interpreted by the reviewers as an adaptive response of the stomach to the physical properties of the test article and were not considered to be of toxicological concern.

b) Organ weights: There were no significant differences in absolute or relative testes weights in P of F_1 males.

c) Histopathology: Treatment-related histopathological findings were limited to the

glandular and nonglandular regions of the stomach. In the nonglandular region, hyperkeratosis, acanthosis, and/or edema were apparent in all treatment groups. In the glandular region, atrophy, congestion, dysplasia, metaplasia, and/or hypertrophic, hyperstaining gastric cells along with an increase in severity of neutrophil infiltration occurred. These changes were interpreted by the reviewers as an adaptive response of the stomach to the physical properties of the test article and were not considered to be of toxicological concern.

B. OFFSPRING

1. Viability and clinical signs: No treatment-related effects on mortality or clinical signs were observed at any time in the F_1 and F_2 litters. Viability indices are shown in Tables 6a and b. Mean litter size was reported for LD 0 only. There was little mortality afterwards, therefore the lack of data for the remainder of lactation was not considered to be a deficiency.

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Table 6a. F_1 generation mean litter size and viability.[a]

Observation	Dose (ppm)			
	0	500	5,000	50,000
Mean litter size (Live)				
Day 0	13.1	13.1	13.7	13.7
Day 1	NR	NR	NR	NR
Day 4[b]	NR	NR	NR	NR
Day 4[c]	NR	NR	NR	NR
Day 7	NR	NR	NR	NR
Day 14[f]	NR	NR	NR	NR
Day 21	NR	NR	NR	NR
Number live pups				
Day 0	315	301	274	273
Day 1	NR	NR	NR	NR
Day 4[b]	311	295	271	260
Day 4[c]	185	175	161	152
Day 7	185	174	161	152
Day 14	185	174	159	152
Day 21	185	174	159	151
Number deaths[d]				
Days 0-4[b]	4	6	3	13
Days 4[c]-21	0	1	2	1
Viability indices (%)				
Livebirth	98.4	99.3	98.2	99.3
Viability (Day 1)	NR	NR	NR	NR
Viability (Day 4)[b]	98.7	98.0	98.9	95.2
Viability (Day 7)	100	99.4	100	100
Viability (Day 14)	100	100	98.8	100
Viability (Day 21)	100	100	100	99.3
Weaning	100	99.4	98.8	99.3
Sex ratio (% male)	NR	NR	NR	NR

a Data extracted from the study report Table 31, pages 91 and 92.
b Before standardization (culling)
c After standardization (culling)
d Calculated by the reviewers.
NR Not reported

Table 6b. F_2 generation mean litter size and viability.[a]

Observation	Dose (ppm)			
	0	500	5,000	50,000
Mean litter size				
Day 0	14.4	14.3	13.9	13.1
Day 1	NR	NR	NR	NR
Day 4[b]	NR	NR	NR	NR
Day 4[c]	NR	NR	NR	NR
Day 7	NR	NR	NR	NR
Day 14	NR	NR	NR	NR
Day 21	NR	NR	NR	NR
Number live pups[d]				
Day 0	317	257	277	302
Day 1	NR	NR	NR	NR
Day 4[b]	313	256	272	300
Day 4[c]	176	144	160	181
Day 7	176	144	159	181
Day 14	176	144	159	181
Day 21	176	144	159	178
Number deaths[d]				
Days 0-4[b]	4	1	5	2
Days 4[c]-21	0	0	1	3
Viability indices (%)				
Livebirth	98.4	98.8	98.6	98.7
Viability (Day 1)	NR	NR	NR	NR
Viability (Day 4)[b]	98.7	99.6	98.2	99.3
Viability (Day 7)	100	100	99.4	100
Viability (Day 14)	100	100	100	100
Viability (Day 21)	100	100	100	98.3
Weaning	100	100	99.4	98.3
Sex ratio (% male)	NR	NR	NR	NR

a Data extracted from the study report Table 35, pages 95 and 96.
b Before standardization (culling)
c After standardization (culling)
d Calculated by the reviewers.
NR Not reported

2. Body weight and gains: Body weights (Table 7) were decreased ($p<0.05$ or 0.01) compared to concurrent controls in high-dose F_1 pups throughout lactation (↓8-26%) in mid-dose pups on LD 14 (↓5%) and mid-dose males on LD 21 (↓5%). In F_2 pups, body weights were decreased ($p<0.05$) in the high-dose pups at LD 14 and 21 (↓9-23%).

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Table 7. Mean F_1 and F_2 pup weights and body weight gains (g).[a]

Lactation Day	Dose (ppm)			
	0	500	5,000	50,000
F_1 litters				
Day 0	6.5	6.6	6.4	6.0*
Day 4[b]	11.4	12.0	10.6	9.5**
Day 4[c]	11.5	11.8	10.6	9.6**
Day 7	18.1	17.5	17.2	15.5**
Day 14	36.5	35.7	34.6*	30.0**
Day 21 Males	59.0	57.6	56.1*	43.7**
Females	55.5	54.5	53.7	41.8**
Gain (0-21)	NR	NR	NR	NR
F_2 litters				
Day 0	6.3	6.3	6.2	6.2
Day 4[b]	10.4	10.0	10.3	10.1
Day 4[c]	10.3	10.1	10.2	10.1
Day 7	17.5	16.8	16.8	16.2
Day 14	35.7	34.6	34.8	32.5*
Day 21 Males	57.9	55.9	55.6	44.7*
Females	54.9	53.2	53.6	43.5*
Gain (0-21)	NR	NR	NR	NR

a Data extracted from the study report Tables 32 and 35, pages 93 and 97.
b Before standardization (culling)
c After standardization (culling)
* or ** Significantly different from control group at $p<0.05$ or 0.01.
NR Not reported

5. Offspring postmortem results:

a). Organ weights: Organs weights were not measured for the F_1 or F_2 pups.

b) Pathology

1) Macroscopic examination: There were no treatment-related findings at necropsy in the F_1 or F_2 pups.

2) Microscopic examination: Histopathology was not performed on any of the F_1 or F_2 pups.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS: At the high-dose, systemic toxicity included mortality, reduced body weight gain in females during gestation, increased water

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consumption in the P animals and the F_1 females and reduced food consumption in the F_1 animals in the first few weeks after weaning. Thickening of the limiting ridge in the stomach was apparent in both generations and sexes. F_1 offspring had reduced body weights throughout lactation and during the first few weeks following weaning. The F_2 offspring also had reduced body weights, but this reduction did not occur until lactation day 7. Histological lesions included papillary acanthosis, diffuse acanthosis, and hyperkeratosis of the nonglandular stomach and hypertrophic, hyperstaining gastric cells, glandular metaplasia, glandular dysplasia, and glandular atrophy of the glandular stomach. At the mid-dose, systemic toxicity was manifested as reduced body weights in F_1 offspring and adults. Microscopic lesions similar to those seen at the high-dose were apparent, but of less severity. There were no differences of toxicological concern in reproductive parameters or offspring survival. The NOAEL for adult systemic toxicity is 500 ppm. The LOAEL is 5,000 ppm. The NOAEL for reproductive toxicity and offspring developmental parameters is 50,000 ppm. The LOAEL was not observed.

B. REVIEWER'S DISCUSSION: In this 2-generation reproduction study, prohexadione calcium (BX-112) was administered continuously to CD rats (26/sex/dose) at nominal dose levels of 0, 500, 5,000 or 50,000 ppm. Based on the concentration analyses, achieved doses were calculated by the reviewers as 0, 355, 3,850, or 38,500 ppm (equivalent to 0, 35.5, 385.0, or 3,850.0 mg/kg/day, respectively, in the P animals and F_1 animals). Exposure to P animals began at 36 days of age and lasted for 56 days prior to mating. F_1 pups selected (26/sex/dose) to produce the F_2 generation were exposed to the same dosage as their parents at PND 21 and continuously throughout the rest of the study. F_1 animals were administered the test article for approximately 63 days prior to mating to produce the F_2 litters. Mating to produce a second F_{2b} generation was not performed. Exposure of all animals to the test material was continuous throughout the study.

The analytical data indicated that the test article formulation was stable.

1. Parental toxicity: There were no treatment-related clinical signs observed in the P or F_1 animals. There were no changes of toxicological concern in food consumption, reproductive performance, gross pathology, testes weights, or histopathology.

In the mid-dose group, two P males and one F_1 female died..

In the high-dose group, two P males died and one female was euthanized *in extremis*. F_1 males had decreased body weights throughout treatment (↓11-30%, $p \leq 0.01$). High-dose F_1 females had reduced body weight for weeks 4-12 and week 15 (↓7-33%, $p \leq 0.05$ or 0.01). Decreases in body weight gains occurred in P females for the overall gestation period (↓17%. $p \leq 0.05$).

The NOAEL for parental systemic toxicity is 355 ppm (35.5 mg/kg/day). The LOAEL for parental systemic toxicity is 3850 ppm (385 mg/kg/day) in males and females based on increased mortality.

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2. Reproductive toxicity: No effects of toxicological concern were observed in the reproductive parameters or function of the adults.

***The reproductive toxicity* NOAEL is ≥38,500 ppm (3850 mg/kg/day). The reproductive toxicity LOAEL is >38,500 ppm (3850 mg/kg/day).**

3. Offspring toxicity: No effects of toxicological concern on mortality or clinical signs were observed at any time in the F_1 and F_2 litters. There were no treatment-related findings at necropsy in the F_1 or F_2 pups.

In the high-dose groups, body weights were decreased ($p<0.05$ or 0.01) in F_1 pups throughout lactation (↓8-26%) and in F_2 pups at LD 14 and 21 (↓9-23%). Body weights were also decreased ($p<0.05$) in the mid-dose groups compared to concurrent controls in F_1 pups on LD 14 (↓5%) and F_1 males on LD 21 (↓5%).

No observations of toxicological significance were made at the low-dose.

The NOAEL for offspring toxicity is 3850 ppm (385 mg/kg/day). The LOAEL for offspring toxicity is 38,500 ppm (3850 mg/kg/day) in males and females based on decreased pup body weight.

The reproductive study in the rat is determined to be **acceptable (§83-4(a), reproduction)** and does satisfy the guideline requirement for a multi-generational reproductive toxicity study in rats.

C. STUDY DEFICIENCIES: No deficiencies were noted.

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